IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Brian Lee Sauer and Andreas Walter Rufer

Serial No.: 09/544,045 Art Unit: 1636

Filed: April 6, 2000 Examiner: William O. Sandals

For: *"METHOD FOR SELECTING RECOMBINASE VARIANTS WITH ALTERED SPECIFICITY"*

United States Patent and Trademark Office
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APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 1-49 in the Office Action mailed April 9, 2003, in the above-identified patent application. A Notice of Appeal was mailed on July 9, 2003. A check in the amount of $160.00 for the filing of this Appeal Brief for a small entity is also enclosed.

It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-1868.

(1) REAL PARTY IN INTEREST

The real party in interest of this application is Oklahoma Medical Research Foundation, the assignee.

(2) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to appellant, the undersigned, or appellant's assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1-49 are pending and on appeal. The text of each claim on appeal, as pending, is set forth in an Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

The claims were last amended in the Amendment mailed May 28, 2002.

(5) SUMMARY OF THE INVENTION

The claimed invention is directed to methods of identifying variant recombinases that mediate recombination at variant recombination sites (claims 1-23) and methods for producing site-specific recombination of DNA (claims 24-49). The methods for identification of variant recombinases comprise, *inter alia*, a) bringing into contact a mutant recombinase, a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase (claims 1 and 24). It is extremely important to note that the first and second sites are both variant recombinant sites, and that the claimed methods are directed to identifying ***variant*** recombinases. ***Variant recombination sites are not recognized by a non-mutant***

recombinase(s), but will undergo recombination in the presence of a mutant recombinase with altered specificity (page 4, lines 1-8).

The claimed methods also comprise making a determination as to whether or not recombination occurs between the first and second recombination sites, and as to whether or not recombination occurs between the third and fourth recombination sites (claims 1 and 24). Recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites. Recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites (page 4, lines 19-23; page 19, lines 7-26).

The recombination sites may comprise recognition sequences and compatibility sequences (page 5, lines 19-29). The recombination frequency between the first and second recombination sites mediated by a non-mutant recombinase may be significantly reduced (page 5, lines 13-20). The sequences of the first and second, and/or third and fourth recombination sites may be identical (page 20, lines 16-18).

The claimed methods may make use of reporter genes, wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by altered expression of a first reporter gene, and wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by altered expression of the

second reporter gene (lines 23-10, bridging pages 4 and 5). The first and second recombination sites may be flanked by a spacer sequence, which interrupts the first reporter gene such that the first reporter gene is not expressed, wherein recombination of the first and second recombination sites excises the spacer sequence, allowing the first reporter gene to be expressed (page 4, lines 28-30). A portion of the first reporter gene may be inverted and flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites results in the inversion of the inverted portion of the first reporter gene, which allows the first reporter gene to be expressed (page 8, lines 6-14; and Figure 11).

Recombination between the first and second recombination sites may prevent expression of the first reporter gene. Recombination of between the third and fourth recombination sites may allow for a second reporter gene to be expressed (page 19, lines 7-26).

The claims are additionally directed to methods for producing site-specific recombination of DNA, comprising contacting a variant recombinase with a DNA sequence comprising a fifth recombination site and another DNA sequence comprising a sixth recombination site, wherein the variant recombinase mediates recombination between the fifth and sixth recombination sites, thereby producing the site specific recombination (page 6, lines 3-4). DNA sequences may be connected by pre-selected DNA segments (page 32, lines 26-28). The recombination sites may have the same orientation and the site-specific recombination of DNA may be a deletion of the pre-selected DNA segment (page 33, lines 1-3). The pre-selected segment may be an undesired marker or trait gene (page 32, lines 3-5 and lines 15-25). The recombination sites may have

opposite orientations so the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment (page 33, lines 3-6).

The substrates for the recombinases may be a gene for a structural protein, an enzyme, or a regulatory molecule (lines 26-1, bridging pages 32-33). Recombination between recombination sites may result in the association of a label with a DNA sequence (page 31, lines 25-28). Recombination may occur in a cell, such as a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism (page 12, lines 25-27). The multicellular organism may be a plant, an animal, or a mammal (page 28, lines 25-28). A DNA sequence may comprise a regulatory nucleotide sequence, wherein expression of the variant recombinase is produced by activating the regulatory nucleotide sequence (page 13, lines 1-20). The recombined DNA may be introduced into a cell (page 29, lines 9-17; and page 31, lines 19-20).

(6) ISSUES ON APPEAL

The issues presented on appeal are:

(1) whether claims 1-47 and 49 are enabled as required by 35 U.S.C. § 112, first paragraph;

(2) whether claims 1-6 and 21 lack novelty under 35 U.S.C. § 102(b) over Miller, et al. *Cell* 20,721-729 (1980) ("Miller");

(3) whether claims 1-6 and 21 lack novelty under 35 U.S.C. § 102(b) over Ackroyd, et al. *J. Mol. Bio.* 216,633-643 (1990) ("Ackroyd"); and

(3) whether claims 1-30, 32-45 and 47-49 are obvious under 35 U.S.C. § 103(a) over Miller or Ackroyd; in view of U.S. Patent No. 5,677,177 to Wahl *et al.* ("Wahl").

(7) GROUPING OF CLAIMS

The claims do not stand or fall together. The claims can be grouped as follows: (1) claim 1, directed, *inter alia*, to a method of identifying variant recombinases comprising contacting a mutant recombinase with a first nucleic acid sequence comprising a first reporter gene and first and second variant recombination sites; (2) claims 2, 4, 7, 8, 10, 11, 13-17, 19-23, directed to further defining the recombination sites; (3) claims 3, 5, 6, 9, 12 and 18, directed to the resultant products of the recombination event; (4) claim 24, directed, *inter alia*, to producing site-specific recombination of DNA comprising, contacting a variant recombinase identified by the method of claim 1 with two DNA sequences, wherein one of the two DNA sequences comprises a first recombination site and the second of the two DNA sequences comprises a second recombination site, wherein the variant recombinase mediates recombination between the sites thereby producing the site specific recombination; (5) claims 25, 26, 27, 28, 29, 30, 32, 33-39, 42, 45 and 47, directed to further defining the nucleic acid sequences in the method to produce site specific recombination (i.e. connecting sequences *via* pre-selected DNA segment(s), recombination sites are variant, orientation of sites, further characterizing the pre-selected DNA segment, DNA sequences harboring label, etc.); (6) claim 31, directed to contacting wild type recombinase, thereby producing site specific recombination between the recombination sites resulting in a deletion of the second pre-selected DNA segment; and (7) claims 40, 41, 43, 44, 46, 48 and 49, directed to compartmentalizing each recombination event (i.e. in a cell; and

further defining the cell). Reasons for this grouping and arguments for the separate patentability of these groups of claims are provided below.

(8) ARGUMENTS

(a) The Claimed Invention

As stated above, the claimed invention is directed to methods of identifying variant recombinases that mediate recombination at variant recombination sites and producing site-specific recombination of DNA. The methods for identification of variant recombinases comprise, *inter alia*, a) bringing into contact a mutant recombinase, a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase. *It is extremely important to note that the first and second sites are both variant recombinant sites.*

(b) Rejections Under 35 U.S.C. § 112, enablement

i. The Legal Standard for Enablement

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art, without undue experimentation (*See, e.g.*, *Genentech, Inc. v. Novo Nordisk A/S*, 108 F3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993); See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v.*

Telectronics, Inc., 857 F.2d 778 (Fed. Cir. 1988); *In re Stephens,* 529 F.2d 1343 (CCPA 1976)). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation (*M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985)). In addition, as affirmed by the Court in *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524 (Fed. Cir. 1987), a patent need not teach, and preferably omits, what is well known in the art.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir. 1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors involved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation 'must not be unduly extensive.' *Atlas Powder Co., v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). There is no requirement for examples.

ii. Claims 1-47 and 49 are enabled as required by 35 U.S.C. § 112, first paragraph

Appellants are claiming methods to identify, and use, variant recombinases. The examiner has rejected the claims solely on the basis that they may read on "gene therapy". The art cited by the Examiner to support his rejections is drawn to the efficacy of gene therapy, a collection of methodologies for the efficient genetic modification of somatic cells to give a particular therapeutic outcome. The cited references point out that it is critical to modify a majority or all of the somatic cells of the organism.

The rejection is completely inappropriate with respect to claims 1-23, drawn solely to a method to identify variant recombinases. These methods do not involve "gene therapy".

The rejection is equally inappropriate as to claims 24-47 and 49. First, there are many *in vitro* applications of the claimed methods which do not require modification of multicellular organism. The examiner has completely ignored this fact.

Second, even if the methodology is used to modify cells in an embryo or other multicellular organism or tissue, modification of the germline of the embryo can be achieved using standard techniques to introduce the variant recombinase and a given DNA so that it will be present in all or most of the cells of the resulting animal. Using standard, well known methods, the claimed methods can be used to place genes at genomic loci, resulting in reliable patterns of gene expression. For example, placement of a loxP reporter DNA at the ROSA26 locus in the mouse gives a reliable and reproducible expression of a reporter gene whose expression changes upon site-specific DNA recombination (see, for example, the reference by

Soriano *et al.*, 1999, *Nat. Genetics* 21:70; submitted with the response mailed on July 9, 2003). Since it is the phenotype of the cell(s) in the organism that is important, and not the phenotype of the organism as a whole, any means to deliver genes into somatic cells would be sufficient to practice the claimed method. For example, delivery of the variant recombinases has been achieved by using genetically modified viruses to express Cre recombinase (see abstract of Badorf *et al.*, *Genesis* 33:119 (2002); submitted with the response mailed on July 9, 2003). Problems associated with gene therapy, for example those outlined in various documents cited by the Examiner, speak to the therapeutic benefit by changing the phenotype of the organism as a whole. However, altering the phenotype of an organism is not necessary to detect a change in recombination "status" in individual cells of that organism. The somatic cell delivery problems associated with gene therapy (host immune problems, efficient DNA delivery to individual cells, etc.) are not applicable when using a transgenic animal approach that gives germline modification.

Third, one skilled in the art would not expect problems in practicing the claimed methods, even in multicellular organisms. Several site-specific recombinases have been shown to catalyze recombination in multicellular organisms following a demonstration that they catalyze recombination in cultured cells. For example Cre in mice (see articles submitted July 9, 2003: Lakso *et al., Proc. Natl. Acad. Sci.* USA 89:6232-6236 (1992); Sauer, *Methods* 14:381-392 (1998); and Metzger *et al.*, 1999, *Curr. Opin. Biotechnol.* 10:470-476 (1999)); Cre in plants (see patent submitted July 9, 2003: U.S. Patent No. 5,658,772 to Odell *et al.*); Flp in mice (see article submitted July 9, 2003: Dymecki, S. *Proc. Natl. Acad. Sci.* USA 93:6191- 6196 (1996));

phiC31 Int in mice (see abstract submitted July 9, 2003: Olivares *et al. Nat. Biotechnol.* 20:1124 (2002).

In summary, the claimed methods are clearly enabled in view of what was commonly known to one of ordinary skill in the art at the time of filing the present application.

(c) Rejections Under 35 U.S.C. § 102

i. The Legal Standard

For a rejection of claims to be properly founded under 35 USC §102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc v Monoclonal Antibodies Inc*, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 US 947 (1987); *Scripps Clinic & Research Found v Genentech Inc*, 18 USPQ2d 1001 (Fed. Cir. 1991). The Federal Circuit held in *Scripps*, 18 USPQ2d at 1010:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. . . *There must be no difference* between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. (Emphasis added)

A reference that fails to disclose even one limitation will not be found to anticipate, even if the missing limitation could be discoverable through further experimentation. As the Federal Circuit held in *Scripps, Id.*:

> [A] finding of anticipation requires that all aspects of the claimed invention were already described in a single reference: a finding that is not supportable if it is necessary to prove facts beyond those disclosed in the reference in order to meet the

claim limitations. The role of extrinsic evidence is to educate the decision-maker to what the reference meant to persons of ordinary skill in the field of the invention, not to fill in the gaps in the reference.

For a prior art reference to anticipate a claim, it must enable a person skilled in the art to practice the invention. The Federal Circuit held that "a §102(b) reference must sufficiently describe the claimed invention to have placed the public in possession of it. . . [E]ven if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling." *Paperless Accounting Inc v Bay Area Rapid Transit Sys.*, 231 USPQ 649, 653 (Fed. Cir. 1986) (citations omitted).

ii. Claims 1-6 and 21 are novel under 35 U.S.C. § 102 (b) over Miller, et al. *Cell* 20,721-729 (1980) ("Miller")

Throughout prosecution, the Examiner has referred to page 725, column 2 (the Discussion) of the Miller reference, wherein lambda integrase activity is manifested under two conditions: in bacterial mutants that fail to support lambda site-specific recombination, and under conditions where the recombinational att sites are altered. However, the appellants' submit that one MUST look to the body of the paper (i.e. the data) in order to properly define "the recombinational att sites" being discussed, and to precisely define which sites are actually being recombined. The appellants respectfully submit that in ALL of the data presented by Miller, recombination proceeds between a wild type site and a variant site. For example, Miller teaches the isolation and characterization of an int protein that is active in a variety of mutants normally defective I lambda site-specific recombination. Lambda-promoted gal expression is dependent

upon the lambda N protein. It should be noted that the secondary sites (i.e. those suggested by the Examiner as being variant) are the same as those used by lambda-int^{+}. Therefore, by definition, *the secondary sites of Miller are not variant recombination sites that are not recognized by non-mutant recombinase* (see page 4, lines 5-8, of the present application, wherein "**the constructs contain variant recombination sites that are not recognized by non-mutant recombinase but will undergo recombination in the presence of a mutant recombinase with altered specificity**" (emphasis added)). With regard to the non-paradigm att sites, the data supplied in column 2 (page 725) is clearly directed to recombination between one variant site and one non-variant site (i.e. "one variant carries the att24 mutation in attL and the other in attR....17% for lambda-attL24-arrR and to 11% for lambda-attL-attR24."). However, the claims on Appeal are directed to, in part, "a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, ***wherein the first and second recombination sites are variant recombination sites***" (emphasis added). In **ALL** of the cases presented in Miller (as data by Miller) recombination proceeds between a wild type site and a variant site. Therefore, the cited passage at page 725, column 2 (the Discussion), wherein lambda integrase activity is manifested under certain conditions, does not pertain to situations wherein **BOTH** sites are altered.

In the Advisory Action (mailed on July 29, 2003), the Examiner refers to a reference, cited in Miller (Shulman and Gottesman, 1973; at page 725, column 2, of Miller), wherein Miller summarizes the reference by stating, "[A] 100 fold reduction in recombination is seen if one or both att sites of λattL-attR carry the att24 mutation." It is important to note that the Shulman and

Gottesman reference was never cited by the Examiner (appellants cannot find any such reference in any submitted "Notice of References Cited" and do not have a copy of this paper). However, Miller fails to teach or suggest **ANYTHING** related to a recombinase that recombines the sites in Shulman and Gottesman.

In the claimed methods, recombination between the first and second recombination sites indicates that **the mutant recombinase** is a variant recombinase **that mediates recombination at variant recombination sites**. There is no teaching in Miller of a variant recombinase with altered specificity (i.e. a variant recombinase that recognizes sequences *that are not recognized* by non-mutant recombinases). Presumably, the 100 fold reduction in the Shulman and Gottesman reference (cited in Miller) is due to the **LACK** of a variant recombinase that **CAN** recombine the altered sites.

iii. Claims 1-6 and 21 are novel under 35 U.S.C. § 102 (b) over Ackroyd, et a. *J. Mol. Bio.* 216, 633-643 (1990) ("Ackroyd")

The Examiner has continually asserted that the abstract of Ackroyd teaches a Tn3 resolvase is mutated by changing an amino acid to a corresponding amino acid from Tn21. The appellants respectfully submit that Ackroyd DOES NOT teach any Tn3 mutant resolvase. The abstract of Ackroyd describes mutating the TN21 resolvase, not Tn3. The Tn3 resolvase that is tested throughout Ackroyd, is WILD-TYPE. Ackroyd teaches the ability of Tn21 resolvase mutants to carry out site-specific recombination between res sites from either Tn21 or Tn3. Nowhere does Ackroyd teach "mutated Tn3 resolvases retain their ability to recombine at the

wild-type Tn3 and are now able to recombine at wild-type Tn12 recombination site" (see page 11 of the Office Action mailed on April 9, 2003).

With further regard to Ackroyd, the transformants that harbor pEAK6 (wild type Tn21) or any of its mutants all yielded approximately the same number of colonies in the presence and absence of tetracycline (see Table 1 and page 638, second column, of Ackroyd). With the exception of the E173L mutant, each of the mutants gave virtually the same levels of recombination with regard to Tn21 res (pAA3) (i.e. the same or reduced activity as wild type). Since the Tn3 resolvase is a wild-type resolvase, the data presented in Ackroyd clearly teaches variant resolvases that recognize DNA not unlike their wild type counterpart(s).

Variant sites, as defined by the claims, are not recognized by non-mutant recombinases (see page 4, lines 5-8, of the present specification). The ability of the Tn21 resolvase and each of the mutants to provide 100% recombination of Rn21 res (E173L = 7%) is a direct contradiction of what the present application teaches. Ackroyd fails to identify ANY mutant recombinase that can recognize ANY NEW recombination site. All sites used in Ackroyd are either wild type Tn12 res sites or wild type Tn3 res sites. Additionally, Ackroyd compares wild type and mutant recombinase activity, on two different sites, in a sequential manner in different cells.

(d) Rejections Under 35 U.S.C. § 103

i. The Legal Standard.

The U.S. Patent and Trademark Office has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Warner et al.*, 379 F.2d 1011, 154 U.S.P.Q. 173, 177 (C.C.P.A. 1967), *In re Fine,* 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988).

In rejecting a claim under 35 U.S.C. § 103, the Examiner must establish a *prima facie* case that: (i) the prior art suggests the claimed invention; and (ii) the prior art indicates that the invention would have a reasonable likelihood of success. *In re Dow Chemical Company,* 837 F.2d 469, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988).

The prior art must provide one of ordinary skill in the art with the motivation to make the proposed modifications needed to arrive at the claimed invention. *In re Geiger*, 815 F.2d 686, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987); *In re Lalu and Foulletier*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). Claims for an invention are not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the application claims. *In re Fritch*, 23 U.S.P.Q.2d, 1780 (Fed. Cir. 1992). *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989). This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication.

The Court of Appeals for the Federal Circuit warned that "the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for showing of the teaching or motivation to combine prior art references." *In re Dembiczak*, 175 F.3d 994 at 999 (Fed. Cir. 1999). While the suggestion to combine may be found in explicit or implicit teachings within the references, from the ordinary knowledge of those skilled in the art, or from the nature of the problem to be solved, the "question is whether there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination. *WMS Gaming, Inc. v International Game Technology*,

184 F.3d 1339 at 1355 (Fed. Cir. 1999). "The range of sources available, however, does not diminish the requirement for actual evidence. That is, the showing must be clear and particular." *In re Dembiczak*, 175 F.3d 994 at 999 (Fed. Cir. 1999). Although with the answer in hand, the "solution" now appears obvious, that is not the test. The references must themselves lead those in the art to what is claimed. And in this case, there is simply no such teaching.

ii. Claims 1-30, 32-45 and 47-49 are not obvious under 35 U.S.C. § 103 (a) over Miller or Ackroyd in view of U.S. Patent No. 5,677,177 to Wahl *et al.* ("Wahl")

Neither of Miller or Ackroyd teach the identification of a mutant recombinase, wherein the **mutant** recombinase recombines first and second sites **that are both variant**. Miller does not teach bringing a mutant Int recombinase together with a pair of mutant att sites. Instead, Miller teaches bringing together a mutant Int recombinase with a pair of att sites, one of which is mutant and the other is wild type. Ackroyd teaches the **sequential** testing of a mutant recombinase with each of two different pairs of recombination sites (i.e. first, test one pair of sites; and then test a second pair of sites. When this was done in cells, Ackroyd used separate cells (i.e. different bacterial strains)). Ackroyd's teachings do not result in the identification of a recombinase that is able to recombine variant recombination sites.

Wahl fails to teach a variant recombinase, or any alteration of substrate specificity. Therefore, even in combination, there is nothing that would lead one skilled in the art to the claimed methods.

(e) The Examiner has failed to individually examine the dependent claims

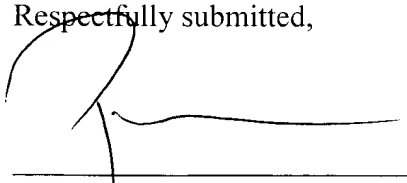
It is well established that each claim must be separately examined for patentability. It is not enough, as here, to look at a single independent claim and reject all claims. No rationale has been presented as to why the subject matter of claims 6 and 21 should be rejected as not being novel in view of the references cited under 35 U.S.C. § 102(b). Expression of reporter genes, and/or the sequences to be recombined that are harbored on separate constructs are completely separate issues with regard to methods of identifying variant recombinases. With regard to enablement issues raised by the Examiner, in addition to the discussion above with respect to claims 1-23 relating not to a method of recombination but to a method of identifying recombinases, nothing has been presented as to why the subject matter of the dependent claims, for example, 16 and 17, are not enabled in view of the specification and what was commonly known in the art at the time of filing the present application. Specific DNA sequences, such as those claimed, and their arrangement within specific constructs (as discussed in the present specification and claimed) present entirely different issues with regard to identifying variant recombinases and directing site-specific recombination. Furthermore, there has been no cited prior art that would make obvious any of the specific nucleotide sequences used in the claimed methods (i.e. spacer sequences, see claim 7; inverted gene segments, see claim 8; reporter genes flanked by recombination sites, see claim 10; connecting DNA sequences *via* pre-selected DNA segments, see claim 26; etc.). These sequences, and their incorporation into the claimed methods, raise very different issues to be considered while reviewing prior art as it relates to the claimed methods.

(9) SUMMARY AND CONCLUSION

The appellants respectfully submit that there is no teaching in the prior art, individually or in combination, of a variant recombinase with altered specificity (i.e. recognizing sequences that are not recognized by non-mutant recombinases). Furthermore, there is no teaching of assessing the recombination of two sites, wherein the two sites are variant. The appellants enclosed a reference by Yoziyanov *et al. Nucleic Acids Res.*30(7): 1656-1663 (2002) with the response and amendment mailed on December 27, 2002. This reference, published after the filing date of the present application, extols the novelty of using a double recombination reporter strategy, as is presently claimed, and provides independent third party evidence of the novelty and non-obviousness of the claimed method. It is also evidence that those skilled in the art believe that such technology is enabled based on no more disclosure than was present in the application on appeal as originally filed.

For the foregoing reasons, Appellant submits that the claims 1-49 are patentable.

Respectfully submitted,



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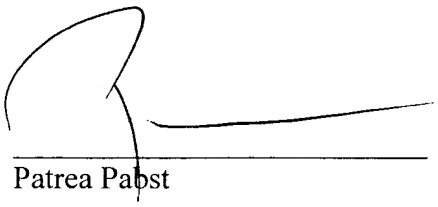

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Date: September 9, 2003

Appendix: Claims On Appeal

1. (original) A method of identifying variant recombinases that mediate recombination at variant recombination sites, the method comprising,

(a) bringing into contact

a mutant recombinase,

a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and

a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase,

(b) determining if recombination occurs between the first and second recombination sites, and determining if recombination occurs between the third and fourth recombination sites,

wherein recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites,

wherein recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites.

2. (previously amended) The method of claim 1 wherein the recombination sites comprise recognition sequences and compatibility sequences,

wherein the recognition sequences of the first and second recombination sites differ from the recognition sequences of the third and fourth recombination sites,

wherein the compatibility sequences of the first and second recombination sites are sufficiently similar to allow recombination between the first and second recombination sites, and wherein the compatibility sequences of the third and fourth recombination sites are sufficiently similar to allow recombination between the third and fourth recombination sites, and

wherein the compatibility sequences of the first and second recombination sites differ from the compatibility sequences of the third and fourth recombination sites such that neither the first nor the second recombination site can be recombined with either the third or the fourth recombination site.

3. (previously amended) The method of claim 1 wherein recombination frequency between the first and second recombination sites mediated by a non-mutant recombinase is significantly reduced.

4. (original) The method of claim 1 or 2 wherein the first and second recombination sites have identical sequences, and wherein the third and fourth recombination sites have identical sequences.

5. (original) The method of claim 1 wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by determining if expression of the first reporter gene is altered, and

wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by determining if expression of the second reporter gene is altered.

6. (original) The method of claim 5 wherein recombination between the first and second recombination sites allows the first reporter gene to be expressed.

7. (original) The method of claim 6 wherein the first nucleic acid sequence further comprises a spacer sequence flanked by the first and second recombination sites, wherein the spacer sequence interrupts the first reporter gene such that the first reporter gene is not expressed, wherein recombination of the first and second recombination sites excises the spacer sequence which allows the first reporter gene to be expressed.

8. (original) The method of claim 6 wherein a portion of the first reporter gene is inverted, wherein the inverted portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the inverted portion of the first reporter gene which allows the first reporter gene to be expressed.

9. (original) The method of claim 5 wherein recombination between the first and second recombination sites prevents expression of the first reporter gene.

10. (original) The method of claim 9 wherein the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites excises the first reporter gene which prevents expression of the first reporter gene.

11. (original) The method of claim 9 wherein a portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the flanked portion of the first reporter gene which prevents expression of the first reporter gene.

12. (original) The method of claim 5 wherein recombination between the third and fourth recombination sites allows the second reporter gene to be expressed.

13. (original) The method of claim 12 wherein the second nucleic acid sequence further comprises a spacer sequence flanked by the third and fourth recombination sites, wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not expressed, wherein recombination of the third and fourth recombination sites excises the spacer sequence which allows the second reporter gene to be expressed.

14. (original) The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not transcribed.

15. (original) The method of claim 13 wherein the second reporter gene encodes a protein, wherein the spacer sequence interrupts the second reporter gene such that the protein encoded by the second reporter gene is not translated.

16. (original) The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene produces an inactive expression product.

17. (original) The method of claim 12 wherein a portion of the second reporter gene is inverted, wherein the inverted portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites

inverts the inverted portion of the second reporter gene which allows the second reporter gene to be expressed.

18. (original) The method of claim 5 wherein recombination between the third and fourth recombination sites prevents expression of the second reporter gene to be expressed.

19. (original) The method of claim 18 wherein the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites excises the second reporter gene which prevents expression of the second reporter gene.

20. (original) The method of claim 18 wherein a portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the flanked portion of the second reporter gene which prevents expression of the second reporter gene.

21. (original) The method of claim 1 wherein the first nucleic acid sequence is a first nucleic acid construct and the second nucleic acid sequence is on a second nucleic acid construct.

22. (original) The method of claim 21 wherein the first nucleic acid construct is an extrachromosomal vector and the second nucleic acid construct is in the genome of a host cell.

23. (original) The method of claim 1 wherein the first and second nucleic acid constructs are on the same nucleic acid construct.

24. (original) A method for producing site-specific recombination of DNA, comprising,

contacting a variant recombinase identified by the method of claim 1 with third and fourth DNA sequences,

wherein the third DNA sequence comprises a fifth recombination site and the fourth DNA sequence comprises a sixth recombination site,

wherein the variant recombinase mediates recombination between the fifth and sixth recombination sites thereby producing the site specific recombination.

25. (previously amended) The method of claim 24 wherein the fifth recombination site, the sixth recombination site, or both, are variant recombination sites.

26. (previously amended) The method of claim 24, wherein the third and fourth DNA sequences are connected by a pre-selected DNA segment.

27. (previously amended) The method of claim 26, wherein the fifth and sixth recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

28. (original) The method of claim 27, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

29. (previously amended) The method of claim 27 further comprising contacting the variant recombinase with a fifth DNA sequence comprising a seventh recombination site, wherein the fourth and fifth DNA sequences are connected by a second pre-selected DNA segment.

30. (previously amended) The method of claim 29 wherein the fifth recombination site is a variant recombination site recognized by the variant recombinase and not by wild type

recombinase, and wherein the sixth and seventh recombination sites are recombination sites recognized by wild type recombinase and by the variant recombinase.

31. (previously amended) The method of claim 30 further comprising, prior to contacting the variant recombinase with the fifth, sixth, and seventh recombination sites, contacting the recombination sites with wild type recombinase, thereby producing site specific recombination between the sixth and seventh recombination sites resulting in a deletion of the second pre-selected DNA segment.

32. (original) The method of claim 29, wherein the second pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

33. (previously amended) The method of claim 26, wherein the fifth and sixth recombination sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.

34. (previously amended) The method of claim 33, wherein the fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

35. (original) The method of claim 33, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

36. (previously amended) The method of claim 24, wherein the fourth and fifth DNA sequences are introduced into two different DNA molecules and the site-specific recombination is a reciprocal exchange of DNA segments proximate to the recombination sites.

37. (previously amended) The method of claim 36, wherein the fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

38. (previously amended) The method of claim 24 wherein the fourth DNA sequence includes a label, wherein recombination between the fifth and sixth recombination sites associates the label with the third DNA sequence.

39. (previously amended) The method of claim 38 wherein the third DNA sequence is a large circular DNA molecule.

40. (original) The method of claim 24 wherein recombination occurs in a cell.

41. (previously amended) The method of claim 40 wherein the variant recombinase is contacted with the third and fourth DNA sequences by introducing into the cell a sixth DNA sequence comprising DNA encoding the variant recombinase.

42. (previously amended) The method of claim 41, wherein the sixth DNA sequence further comprises a regulatory nucleotide sequence and expression of the variant recombinase is produced by activating the regulatory nucleotide sequence.

43. (original) The method of claim 40, wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

44. (original) The method of claim 43, wherein the multicellular organism is a plant, an animal, or a mammal.

45. (previously amended) The method of claim 40, wherein the third and fourth DNA sequences are connected by a pre-selected DNA segment, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

46. (original) The method of claim 45, wherein the cell is a multicellular organism.

47. (original) The method of claim 45, wherein the pre-selected segment is an undesired marker or trait gene.

48. (original) The method of claim 24, wherein the variant recombinase is contacted with the recombination sites *in vitro*.

49. (original) The method of claim 48, wherein the method further comprises introducing the recombined DNA into a cell.

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